Laboratory Detection of *Chlamydia trachomatis*

Chlamydia trachomatis infection is the leading cause of sexually transmitted diseases in the Western world. Lower genital tract infections in females are frequently asymptomatic and therefore undetected. Infections by *C trachomatis* can involve the upper genital tract as well, leading to tubal scarring and infertility. Neonatal infections due to exposure at birth are also well-established complications. For these reasons, routine screening is recommended for those most at risk of developing a *C trachomatis* infection—sexually active adolescents and women in their early twenties and those with new sexual partners.

Tests for the laboratory detection of *C trachomatis* are based on detecting antigen, nucleic acid, or viable organisms. Since there are major differences in the performance, sensitivity, and specificity of these tests, it is important to understand these distinctions.

The first laboratory test available, the culture, was until recently the gold standard of *Chlamydia* testing. Its specificity of 100% makes it the only recommended test for children or victims of sexual abuse. Culture should detect even a single viable *Chlamydia* organism, but its sensitivity can be compromised by variables such as loss of viability upon transport or storage, differences in susceptibilities of cell lines, and stains used in the culture technique. Furthermore, there is no standard culture method, so results vary widely between laboratories.

The first commercially available assays were based on the detection of chlamydial antigens using polyclonal and/or monoclonal antibodies that recognize either the major outer membrane protein or lipopolysaccharide of the organism. Today, antigen detection tests range from a 20-minute paper- or wafer-based assay to a 3- to 4-hour enzyme immunoassay (EIA). The specificity of the assays is, with few exceptions, acceptable (>98%). Reported sensitivities of these tests vary tremendously, neutralizing any comparison based on literature review. Most evaluations of the antigen-based assays have used culture as a gold standard but, since the sensitivity of the culture method can vary, the apparent sensitivity of the antigen detection method fluctuates. When DNA amplification methods are run in parallel with culture, the sensitivity of the antigen-based assays ranges between 50 and 70%. Therefore, while these assays may be convenient in terms of time, simplicity, and cost, many are too insensitive for routine screening. DNA hybridization methods, which are labor-intensive, have proven to yield sensitivities and specificities similar to the antigen-based methods.

The most sensitive means of *Chlamydia* detection so far are the commercially available nucleic acid amplification–based assays, such as polymerase and ligase chain reactions. They are estimated to be 10 to 20% more sensitive than culture. A great advantage is that urine has been shown to be an acceptable specimen for detecting *C trachomatis*. This is a significant advance, especially for testing males, since it makes a urethral swab unnecessary. These assays are more expensive than the antigen detection assays, however. They are also more technically demanding—they require sophisticated equipment and an average of 4–5 hours. Inhibition of the assay has been reported, but future generations of the tests promise to improve on these problems.

In summary, antigen detection methods, while easy to perform and readily available, have low sensitivity. Due to the technical requirements of DNA amplification, the most sensitive method for *Chlamydia* detection, the test is generally not found in small laboratories but in more centralized testing facilities. Culture, due to its variability and tight restrictions of specimen collection and transportation, is impractical for most small laboratories but can be found in large teaching hospitals and reference laboratories.

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Diseases Associated With the Major Histocompatibility Complex

THE MAJOR HISTOCOMPATIBILITY COMPLEX (MHC) is a genetic region that encodes for class I and class II molecules and a variety of other proteins. The class I and class II molecules are human leukocyte antigen (HLA) proteins and are essential for the immune system to recognize and respond to foreign antigens. This region of the genome is diverse, with hundreds of alleles of the different HLA genes. Because of its importance in organ transplantation, the MHC locus has been the focus of many studies; many of the alleles are known. Other than HLA antigens, the MHC encodes for a variety of other proteins including proteins from the complement system and tumor necrosis factor (TNF).

Some HLA alleles have been found to be correlated with specific diseases, so determining a patient's HLA type is useful in diagnosing a disease, diagnosing a variant of a disease, predicting a person's susceptibility to a disease, or predicting the course of a disease. There are several explanations for the predictive value of HLA types. Many infectious and autoimmune diseases are influenced by the immune response. Different HLA types may be associated with differences in immune responses

and may make an individual susceptible or resistant to a particular disease. Also, the MHC region is densely packed with genes, many of which are important for function. Hence, mutations in this region of the genome are more likely to result in a disease than mutations in a region of the genome that has very few coding regions. Furthermore, the MHC region may be a "hot spot" for mutagenesis. Finally, the polymorphic nature of this region means that a mutation in or near the MHC complex is likely associated with a specific HLA type that is not found in many people. Hence, specific HLA types can serve as markers that are linked with defective genes in this region.

While diseases have been known to be correlated with specific HLA loci for some years, recent advances in molecular biology have resulted in the discovery of many more disease associations. Classic serologic techniques define an HLA type by reactivity of an antibody or antisera with HLA molecules. DNA analysis has shown that one classic HLA type can be further subdivided into a set of alleles with slightly different sequences. These alleles are known as subtypes of an HLA type. If a disease is associated with only one particular HLA subtype, then studies employing classic serologic techniques might fail to establish a statistically significant correlation between the disease and HLA markers. In these cases, molecular techniques can determine HLA subtypes and might establish that one HLA subtype is associated with the disease.

HLA studies can help investigators classify diseases. Juvenile rheumatoid arthritis (JRA), for example, can have several presentations. Researchers proposed classifying JRA into various subtypes based on clinical features and then found that different JRA subtypes correspond with different HLA subtypes. This provides evidence that different forms of JRA arise from different pathophysiologic mechanisms and may be responsive to different therapeutic interventions.

Diseases that are associated with specific HLA types or subtypes include ankylosing spondylitis, type 1 diabetes mellitus, Behçet's disease, celiac disease, Grave's disease, Hashimoto's thyroiditis, Hodgkin's disease, idiopathic membranous nephropathy, idiopathic nephrotic syndrome, multiple sclerosis, narcolepsy, C2 deficiency, C4 deficiency, congenital adrenal hyperplasia, idiopathic hemochromatosis, gluten-sensitive enteropathy, pemphigus vulgaris, cicatrical pemphigoid, Goodpasture's syndrome, juvenile rheumatoid arthritis, and rheumatoid arthritis. In addition, susceptibility to malaria infection and rapid or slow progression of HIV infection are associated with specific HLA types.

For most diseases, molecular techniques that determine HLA subtypes provide the most useful information. These studies can be complicated, however, because in some diseases, such as type 1 diabetes, certain HLA subtypes (DQA1*0301, for example) are associated with an increased risk of contracting the disease but other subtypes (DQA1*0102, for example) are associated with a decreased risk of contracting the disease.

Some of the diseases with the strongest associations with specific HLA genotypes in the general population are ankylosing spondylitis, narcolepsy, and celiac disease. HLA typing can be useful in many other diseases, however. Often when a family member has a disease, the family and physician want to know the likelihood that other family members will develop the disease. In these cases, comparing the HLA type of the proband with relatives can provide useful information even if the disease does not have a strong correlation with particular HLA subtypes in the general population. Also, in specific clinical situations, HLA testing may help refine a differential diagnosis or predict the progression of an illness.

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Kaposi's Sarcoma–Associated Herpesvirus (KSHV): A New Viral Pathogen Associated With Kaposi's Sarcoma, Primary Effusion Lymphoma, and Multicentric Castleman's Disease

KAPOSI'S SARCOMA–ASSOCIATED HERPESVIRUS (KSHV), also known as human herpesvirus 8 (HHV-8), is a member of the gammaherpesvirinae subfamily of herpesvirus that are characterized by the ability to replicate in lymphoblastoid cells. KSHV is related to Epstein-Barr virus (EBV) and herpesvirus saimiri (HVS) and is the first member of the genus *Rhadinovirus* known to infect humans. Viral DNA was first discovered in Kaposi's sarcoma lesions by Chang and Moore, who used representational difference analysis to identify KS330Bam and KS631Bam fragments. As shown by ELISA for HHV-8 antibody, the prevalence of KSHV is much lower than other human herpes viruses (EBV, HHV-6, cytomegalovirus, herpes simplex virus 1). Less than 20% of normal adult donors and 33% of HIV-negative homosexual men have antibodies to KSHV. Both HIV-positive and -negative patients with Kaposi's sarcoma have antibodies to KSHV (titer >1280 in most cases), and seroconversion has been documented before the development of Kaposi's sarcoma. KSHV also is now known to be associated with a new lymphoma subtype, primary effusion lymphoma, and with lymphoid proliferations resembling angioimmunoblastic lymphadenopathy with dysproteinemia (AILD) and multicentric Castleman's disease.

Primary effusion lymphoma occurs predominantly in HIV-infected male patients; the primary symptom is lym-